Claim 42. (New) A plant cell comprising a recombinant DNA construct of claim 40.

Claim 43. (New) A recombinant DNA construct for expression of a nitrite reductase gene in a plant cell, wherein said construct comprises a promoter functional in a plant cell operatively linked to a nucleic acid molecule encoding a nitrite reductase protein having at least 70 percent sequence identity to SEQ ID NO: 11926 over the entire length of said protein.

Claim 44. (New) The recombinant DNA construct of claim 43, wherein said nucleic acid molecule encodes a nitrite reductase protein having at least 90 percent sequence identity to SEQ ID NO: 11926 over the entire length of said protein.

Claim 45. (New) The recombinant DNA construct of claim 43, wherein said nucleic acid molecule encodes a nitrite reductase comprising SEQ ID NO: 11926.

Claim 46. (New) A plant cell comprising a recombinant DNA construct of claim 43.

Remarks

The present application contains claims 1, 8, 11-13, 17-19, 29 and 38-39 and newly added claims 40-46 directed to encoding sequences for a novel nitrite reductase and related proteins, and to recombinant constructs and cells comprising such sequences.

Objection to Specification

The specification was rejected for containing embedded hyperlinks, for example at page 39 lines 4 and 14. The objection is believed obviated by Applicants' submission herein of substitute pages 18 and 39 in which the hyperlinks have been removed.

Rejection of Claims under 35 U.S.C. § 112, First Paragraph – Lack of Enablement

Claims 1, 8, 11-13, 17-19, 29, 38 and 39 were rejected as allegedly directed to subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the claimed sequence. The Patent Office's rejection was based on failure of the claims to recite a particular biological activity for the claimed sequence.

Applicants assert that recitation of the biological activity for a claimed sequence is not required for claims to be enabled where the specification, as here, teaches the activity and teaches one how to make and use such a sequence. In addition to the description provided at page 54 of

the application and acknowledged by the Examiner, Applicants respectfully direct the Examiner's attention to page 9, line 21 to page 10, line 8, where specific uses of the claimed sequence are described.

The above notwithstanding, Applicants have amended independent claims 11, 38 and 39 to specify that the claimed nucleic acid molecule encodes nitrite reductase and respectfully request that the 35 U.S.C. § 112, first paragraph rejection of the claims for lack of enablement be withdrawn.

Rejection of Claims under 35 U.S.C. § 112, First Paragraph – Lack of Written Description

Claims 1, 8, 11-13, 17-19, 29, 38 and 39 were rejected. The Patent Office asserts that the subject matter was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s) had possession of the claimed invention at the time the application was filed.

The Patent Office accepts that SEQ ID NO:4369 and sequences encoding SEQ ID NO:11926 meet the written description requirement. Applicants note that claim 11 as presently amended, and claims 8, 1, 17-19, dependent thereon, are directed to sequences falling within the accepted scope and respectfully request that the rejection be withdrawn.

The Patent Office also asserts, that the claims encompass gene sequences that do not meet the written description requirement because of open claim language (comprising, comprises and having), and that, for example, claims 38 and 39 which use 70% and 90% identity could contain additional sequences on either end of SEQ ID NO:4639. Applicants respectfully traverse the Patent Office's position that open claim language somehow runs afoul of the written description requirement. Clearly, nucleic acid molecules comprising additional sequences beyond those of the nitrite gene sequences of the present invention, such as provided, for example, in claim 8, are elements of Applicants' invention and fully supported by the specification. The Examiner's attention is respectfully drawn to, for example, page 20, line 27 through page 23, line 17. Applicants note that claims 38 and 39 have been amended to clarify that the claimed percent sequence identity is over the entire coding region of SEQ ID NO:4639.

In view of the above comments and the amendments to the claims, Applicants respectfully request that the 35 U.S.C. § 112, first paragraph rejection of the claims for lack of written description be withdrawn.

Rejection of Claims under 35 U.S.C. § 112, Second Paragraph

Claims 1, 8, 11-13, 17-19 and 29 were rejected as being indefinite for failing to particularly point out and distinctly claim the invention.

The rejections of these claims are believed avoided by the above amendments to the claims and Applicants respectfully request that the rejections be withdrawn.

Rejection of Claims under 35 U.S.C. § 102(b)

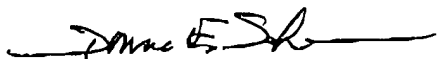
Claims 1, 8, 11, 17, 19, 29, 38 and 39 were rejected as being anticipated by Darwin *et al.* (1993). The phrase "*the* sequence" in claim 11 was viewed by the Patent Office as being undefined as to whether this referred to the entire sequence or a fragment of the sequence.

Applicants note that the amendments to claims 11, 38 and 39 obviate this rejection and respectfully request that the rejection be withdrawn.

CONCLUSION

Applicants submit that the instant application is now in condition for allowance and early notice to this effect is solicited. If in the opinion of the Examiner a telephone conference would expedite prosecution of the instant application, the Examiner is invited to call the undersigned at (860) 572-5262.

Respectfully submitted,



Donna E. Scherer
Agent for Applicants
Reg. No. 34,719

Marked Up Version f the Full Set of Claims

Claim 1. The substantially purified nucleic acid molecule of Claim 11 wherein said nucleic acid molecule comprises SEQ ID NO: 4639.

Claim 8. The substantially purified nucleic acid molecule according to claim 11 wherein said nucleic acid molecule further comprises nucleic acid sequences comprising one or more of a promoter region or regulatory region or parts of said regions.

Claim 11. (Twice Amended) A substantially purified nucleic acid molecule encoding a [nitrate pathway protein] nitrite reductase comprising [the sequence of] SEQ ID NO: 11926.

Claim 12. (Twice Amended) A substantially purified first nucleic acid molecule which is complementary to [a] the entire sequence of the nucleic acid molecule of claim 1, wherein said first nucleic acid molecule and said nucleic acid molecule of claim 1 hybridize to one another [with sufficient stability to remain annealed to one another] under at least low stringency conditions of washing with a salt solution having a concentration of about 2.0 X sodium chloride/sodium citrate (SSC) at 50°C.

Claim 13. The substantially purified first nucleic acid molecule according to claim 12 wherein said stringency conditions are at least 0.2 X SSC at 50°C.

Claim 17. (Thrice Amended) A transformed cell or organism having an exogenous nucleic acid molecule which comprises:

(a) a promoter region which functions in said cell or organism to cause the production of a mRNA molecule; [which] wherein said promoter region is linked to

(c) a nucleic acid molecule of claim 11.

Claim 18. A transformed cell or organism of claim 17 wherein said nucleic acid molecule in (b) is linked to a 3' untranslated sequence that functions in said cell or organism to cause termination of transcription.

Claim 19. The transformed cell or organism according to claim 17 which is selected from the group consisting of a bacterial cell, plant cell, plant, mammalian cell, mammal, fish cell, fish, bird cell, bird, fungal cell and fungus and wherein said mRNA encodes a protein in said cell.

Claim 38. (Amended) A substantially purified nucleic acid molecule encoding nitrite reductase [a nitrate pathway protein], wherein said nucleic acid molecule has at least [about] 70 percent identity to the entire sequence of SEQ ID NO: 4639.

Claim 39. (Amended) [A] The substantially purified nucleic acid molecule [encoding a nitrate pathway protein,] of claim 38, wherein said nucleic acid molecule has at least [about] 90 percent identity to the entire sequence of SEQ ID NO: 4639.

Claim 40. (New) A recombinant DNA construct for expression of a nitrite reductase gene in a plant cell, wherein said construct comprises a promoter functional in a plant cell operatively linked to a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising the entire sequence of SEQ ID NO:4639 under at least low stringency conditions of washing with a salt solution having a concentration of about 2.0 X sodium chloride/sodium citrate (SSC) at 50°C.

Claim 41. (New) The recombinant DNA construct of claim 40, wherein said stringency conditions are at least 0.2 X SSC at 50°C.

Claim 42. (New) A plant cell comprising a recombinant DNA construct of claim 40.

Claim 43. (New) A recombinant DNA construct for expression of a nitrite reductase gene in a plant cell, wherein said construct comprises a promoter functional in a plant cell operatively linked to a nucleic acid molecule encoding a nitrite reductase protein having at least 70 percent sequence identity to SEQ ID NO: 11926 over the entire length of said protein.

Claim 44. (New) The recombinant DNA construct of claim 43, wherein said nucleic acid molecule encodes a nitrite reductase protein having at least 90 percent sequence identity to SEQ ID NO: 11926 over the entire length of said protein.

Claim 45. (New) The recombinant DNA construct of claim 43, wherein said nucleic acid molecule encodes a nitrite reductase comprising SEQ ID NO: 11926.

Claim 46. (New) A plant cell comprising a recombinant DNA construct of claim 43.

PTO/SB/22 (10-00)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): 38-10(15849)B

In re Application of	Barry S. Goldman et al.
Application Number	09/902,540
Filed	07/10/2001
For	Myxococcus xanthus genome sequences and uses thereof
Group Art Unit	1631
Examiner	Carolyn L. Smith

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

- ☐ One month (37 CFR 1.17(a)(1)) $_____
- ☑ Two months (37 CFR 1.17(a)(2)) $ 410
- ☐ Three months (37 CFR 1.17(a)(3)) $_____
- ☐ Four months (37 CFR 1.17(a)(4)) $_____
- ☐ Five months (37 CFR 1.17(a)(5)) $_____

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee amount shown above is reduced by one-half, and the resulting fee is: $_____.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Commissioner has already been authorized to charge fees in this application to a Deposit Account.

☑ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number **134125.**
I have enclosed a duplicate copy of this sheet.

I am the ☐ applicant/inventor

☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

☑ attorney or agent of record.

☐ attorney or agent under 37 CFR 1.34(a). Registration number if acting under 37 CFR 1.34(a)_____.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

May 27, 2003
Date

Signature

Donna E. Scherer, Reg. No. 34,719
Typed or printed name

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☑ Total of 1 forms are submitted.

reverse complement reading frames, and then compares the six translations against a protein sequence database (*e.g.* the non-redundant protein (*i.e.*, nr-aa) database maintained by the National Center for Biotechnology Information as part of GenBank and available at the web site: www.ncbi.nlm.nih.gov). BLASTX is run with the *Myxococcus xanthus* contigs and singletons represented by SEQ ID NO:1 through SEQ ID NO:1849 as queries against the GenBank non-redundant protein data library identified as "nr-aa". To identify genes solely by BLASTX, the minimum BLASTX E value is set at 1E-08.

Since homology-based methods may overlook genes unique to *Myxococcus xanthus*, for which homologous nucleic acid molecules have not yet been identified in databases, gene prediction programs are also used. Additional *M. xanthus* genes with no known homologs under the above BLASTX analysis parameters were predicted using the GeneMark sequence analysis program (Borodovsky *et al. Computers & Chemistry 17*:123-133 (1993)). GeneMark is available from Gene Pro (Atlanta, GA) or from Georgia Tech University (*e.g.* at the web site (see www.genemark.biology.gatech.edu/GeneMark for details). GeneMark calculates the probability of a gene being present based on the presence of a gene-like 'grammer' in the target DNA sequence (*i.e.*, start and stop signals, and a significant open reading frame) and statistical analyses of protein-coding potential through biases in putative codon usage. GeneMark uses inhomogeneous Markov chain models derived from comparisons of known coding and non-coding sequences to predict the presence of protein-coding regions. The GeneMark program is "trained" with *M. xanthus* characteristics. Predicting full-length genes is comprised by point mutations in the unfinished contigs, as well as by the short length of contigs relative to the typical length of a gene. Due to the errors found in the full-length gene predictions by GeneMark, inclusion of GeneMark-predicted genes is limited to those genes and ORFs of partial genes whose probabilities are above the threshold of p. > 0.5.

The results of the homology based and predictive analysis methods were merged into a single set of predicted coding regions, and their most probable translation. In setting criteria for confidence of gene prediction, a "high" BLASTX match as used herein means a match having a BLASTX Bit Score as provided in Table 1 of greater than 150; a medium BLASTX Bit Score is 100 to 150; and a low BLASTX Bit Score is less than 100. "Bits" refers to information content, and the score in the "Bits" column indicates the amount of information in the hit. A higher

core sequences which can affect promoter strength. Such additional regulatory sequences may be located upstream of, downstream of, or between core promoter elements. Examples of additional regulatory elements include UP elements (-40 upstream region) and DSR elements (region immediately downstream of the transcription start site).

In a preferred embodiment, the promoter of the present invention is present in a recombinant construct and located upstream of a nucleic acid sequence for expression in *M. xanthus* cells, including nucleic acid sequences that encode an *M. xanthus* protein homolog or fragment thereof. For the most part, the promoters of the present invention will be located in contig sequences which generally represent longer nucleic acids than do singleton sequences of the present invention. Contigs in SEQ ID NO:1 through SEQ ID NO: 1849 are recognized as those sequences whose designations begin with MYX10C, as opposed to singletons whose designations begin with MYX10S.

DNA Replication Elements

The present invention further encompasses *Myxococcus xanthus* DNA replication elements, such as the origin of replication from which replication proceeds, and the terminus, or *ter* site on the circular chromosome. (Marians, *Annu. Rev. Biochem 61*:673-719 (1992)). The origin or replication may be recognized by the presence of conserved DNA structures Eckdahl *et al.*, *Nucleic Acids Res. 18*:1609-12 (1990); Moriya *et al.*, *Saibo Kogaku 15*:13-22 (1996); *Network Sci.* [Electronic Publication] (1995), 1(4, Avail. URL: www.awod.com/netsci/Issues/Oct95/feature4.html). As increased gene dosage has been suggested to occur near origin of replication and *ter* sites under certain doubling time conditions, identification of such sites is useful for use for insertion of recombinant DNA constructs for expression in *Myxococcus* cells.

Polypeptides

Other aspects of this invention comprise one or more of the polypeptides, including proteins or peptide molecules, encoded by a Myxococcus coding region of this invention or fragments thereof or homologs thereof. Coding regions and the encoding protein or peptide molecules of the present invention can be identified using known protein or peptide molecules as a target sequence or target motif, for example using BLAST programs as described herein. In a preferred embodiment the protein or fragment molecules of the present invention are derived